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Studies on the bio-safety of insecticides to *Chrysoperla zastrowi sillemi* (Esben-Peterson) (Neuroptera: Chrysopidae) under labarotary conditions

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Abstract
Studies on bio-safety of insecticides to *Chrysoperla zastrowi sillemi* (Esben-Peterson) (Neuroptera: Chrysopidae) second instar grubs and adults were conducted under the laboratory conditions at ARS, Daharwad, Karnataka during the period 2020-21. The results of the experiment indicated that among the fourteen insecticides treated,, the lowest toxicity was found in the treatment of azadirachtin 0.03% EC followed by spinetoram 11.7% SC, flonicamid 50% WG. Whereas, the highest toxicity was found in the treatment imposed with the acephate 50% + imidacloprid 1.8% SP followed by imidacloprid 6% + lambda-cyhalothrin 4% SL, lambda-cyhalothrin 5% EC, profenofos 50% EC against second instar grubs. At 24 hrs of exposures to insectides for adults revealed that, lowest toxicity was recorded in the treatment of azadirachtin 0.03% EC followed by spinetoram 11.7% SC, diafenthiuron 50% WP, while the highest toxicity was recorded in treatment of profenofos 50% EC fallowed by lambda-cyhalothrin 5% EC and acephate 50% + imidacloprid 1.8% SP.

Keywords: Insecticides, *Chrysoperla zastrowi sillemi*, Toxicity, grubs and adults

Introduction
The green lacewing, *Chrysoperla zastrowi sillemi* also known as golden eyes and aphid lions is an important predator belonging to order Neuroptera. Its larvae are voracious and polyphagous predators, feeding on jassids, psyllids, aphids, coccids and mites. *Chrysoperla* sp. received much attention of farmers as well as researchers as a potential biological control agent due to its broad host range. It is widely distributed and easily amenable to mass multiplication under laboratory conditions. Due to growing environmental and economic concerns involved in the use of pesticides, there is dire need to develop alternate measures for the management of sucking pests. Pesticides lead to many serious problems like pollution, health hazards, biodiversity threat, pest resurgence, pest resistance and secondary pest out-breaks in ecosystem (Bellows, 200l) [3]. Biological control is an effective means of achieving insect control in crop ecosystem. Conservation natural control, in which beneficial fauna are preserved in the agro-ecosystem, has been considered as an element progressively important in Integrated Pest Management (IPM) programs. Effectiveness of *Chrysoperla* sp. as biological control agent had been demonstrated in the crops. Conservation natural control, in which beneficial fauna are preserved in the agro-ecosystem, has been considered as an element progressively important in Integrated Pest Management (IPM) programs (Reddy, 20l6) [7].

Material and methods
Toxicity of insecticides against larvae of *C. zastrowi sillemi*
The experiment was conducted with 14 treatments and 4 replications in Completely Randomized Design. Formulated products of 14 insecticides as described above along with control (water spray) were used to determine their contact toxicity against second instar larvae of *C. zastrowi sillemi*. The relative toxicity of insecticide was tested by using the thin residual film method as suggested by PDBC, Bangalore (Anon., 1994) [2]. For preparing insecticide film, vials were treated by dipping them in respective insecticide solutions, followed by drying under fan for 15 minutes.

Ten numbers of second instar larvae of *C. zastrowi sillemi* were released in each treated vial and allowed to remain in contact with insecticide film for 45 minutes. The set up was replicated four times thus, for each insecticides there was five concentrations, replicated four times. Thereafter, larvae were transferred individually to fresh vials containing *Corcyra* eggs that was serve as food. The observation on larval mortality were recorded at 24, 48 and 72 hours after exposure to insecticide treatments. The per cent corrected mortality was calculated by using Abbott's formula (Abbott, 1925) [1] as under.

$$\text{Corrected mortality (\%)} = \frac{[\%\ \text{mortality (Treatment)} - \%\ \text{mortality (control)}]}{[100 - \%\ \text{mortality (control)}]} \times 100$$

Toxicity of insecticides against adults of *C. zastrowi sillemi*

The toxicity of above insecticides were also tested against adult (2-days old) stage of *C. zastrowi sillemi* with poison food technique as adopted by Rezaei *et al*., (2007) [8] and Sabry and Sayed (2011) [9]. Ten number of healthy adults of *C. zastrowi sillemi* were released in each plastic jar of dimension 20 × 12 cm covered with muslin cloth tied in position with rubber band for each treatment. These adults in four replicates were fed on sugar solution of 10% contaminated with tested concentration of insecticides under study. In the control treatment, the adults were fed with uncontaminated food. All jars were incubated at room temperature and inspected after 24 hour and the number of adults died were recorded in each treatment and replication. The corrected mortality of adults in each treatment were estimated as per Abbott's formula described above and the subjected to probit analysis.

Table 1: Toxicity response of insecticides against grubs of *C. zastrowi sillemi* at 24 hrs after exposure

Insecticides	LC_{50} (ppm or g or ml/L)	Fiducial limit		Regression equation	Slope ± S.E.	χ^2
		Lower	Upper			
Acetamiprid 20% SP	268.96 (0.26)	179.52	500.15	Y=2.55+1.05X	1.05 ± 0.22	0.23
Diafenthiuron 50% WP	762.52 (0.76)	388.58	1973.51	Y=3.40+1.70X	1.7 ± 0.25	0.32
Flonicamid 50% WG	824.54 (0.82)	434.93	2844.75	Y=3.07+1.02X	1.02 ± 0.27	0.38
Fipronil 5% SC	740.56 (0.74)	487.64	2709.87	Y=3.89+1.37X	1.37 ± 0.25	0.68
Acephate 75% SP	253.63 (0.25)	195.29	491.84	Y=2.55+0.9X	0.9 ± 0.23	0.15
Spinetoram 11.7% SC	1187.98 (1.18)	548.31	11903.35	Y=2.20+1.67X	1.67 ± 0.23	0.21
Profenofos 50% EC	241.75 (0.24)	182.27	330.50	Y=3.88+0.97X	0.97 ± 0.26	1.31
Imidacloprid 17.8% SL	724.50 (0.72)	449.09	2302.48	Y=2.86+1.97X	1.97 ± 0.25	0.55
Lambda-cyhalothrin 5% EC	220.06 (0.22)	129.36	598.35	Y=2.19+0.88X	0.88 ± 0.22	0.03
Azadirachtin 0.03% EC	8142.43 (8.14)	7848.33	15592.37	Y=8.97+2.17X	2.17 ± 0.60	0.29
Imidacloprid 6% + Lambda-cyhalothrin 4% SL	215.99 (0.21)	136.05	453.30	Y=2.61+0.98X	0+98 ± 0.23	0.43
Acephate 50% + Imidacloprid 1.8% SP	173.90 (0.17)	109.09	269.27	Y=2.54+0.88X	0.88 ± 0.20	0.28
Fipronil 40% + Imidacloprid 40% WG	323.60 (0.32)	225.33	530.70	Y=3.05+1.61X	1.16 ± 0.26	0.86
Pyriproxyfen 8.0% + Diafenthiuron 30% SE	504.36 (0.50)	337.37	954.77	Y=3.02+1.07X	1.07 ± 0.23	0.39

Table 2: Toxicity response of insecticides against grubs of *C. zastrowi sillemi* at 48 hrs after exposure

Insecticides	LC_{50} (ppm or g or ml/L)	Fiducial limit		Regression equation	Slope ± S.E.	χ^2
		Lower	Upper			
Acetamiprid 20% SP	178.91 (0.17)	108.08	328.17	Y=1.95+0.86X	0.86 ± 0.21	0.03
Diafenthiuron 50% WP	462.41 (0.46)	282.13	1126.23	Y=3.06+1.14X	1.14 ± 0.23	0.02
Flonicamid 50% WG	445.24 (0.44)	281.06	1161.73	Y=2.69+1.97X	1.97 ± 0.25	0.27
Fipronil 5% SC	412.07 (0.41)	347.63	1087.41	Y=3.54+1.26X	1.26 ± 0.27	0.29
Acephate 75% SP	176.36 (0.17)	123.39	262.49	Y=2.72+1.21X	1.21 ± 0.22	0.10
Spinetoram 11.7% SC	624.48 (0.62)	451.53	934.53	Y=2.88+1.01X	1.01 ± 0.23	0.21
Profenofos 50% EC	107.42 (0.10)	63.09	150.22	Y=2.77+0.98X	0.98 ± 0.25	0.31
Imidacloprid 17.8% SL	348.28 (0.34)	267.29	978.07	Y=3.13+1.20X	1.20 ± 0.24	0.57
Lambda-cyhalothrin 5% EC	119.59 (0.11)	72.60	190.25	Y=2.12+0.96X	0.96 ± 0.21	0.16
Azadirachtin 0.03% EC	6671.59 (6.0)	5478.68	8584.09	Y=10.07+2.5X	2.5 ± 0.59	0.45
Imidacloprid 6% + Lambda-cyhalothrin 4% SL	106.26 (0.11)	72.39	184.23	Y=2.03+0.98X	0.98 ± 0.22	0.03
Acephate 50% + Imidacloprid 1.8% SP	97.00 (0.09)	59.39	138.99	Y=2.49+1.19X	1.19 ± 0.20	0.25
Fipronil 40% + Imidacloprid 40% WG	259.92 (0.25)	156.05	451.08	Y=2.52+1.14X	1.14 ± 0.26	0.10
Pyriproxyfen 8.0% + Diafenthiuron 30% SE	321.64 (0.32)	216.80	559.80	Y=2.43+1.30X	120 ± 0.26	0.35

Table 3: Toxicity response of insecticides against grubs of *C. zastrowi sillemi* at 72 hrs after exposure

Insecticides	LC_{50} (ppm or g or ml/L)	Fiducial limit		Regression equation	Slope ± S.E.	χ^2
		Lower	Upper			
Acetamiprid 20% SP	124.45 (0.12)	107.42	273.71	Y=2.38+1.14X	1.14 ± 0.22	0.24
Diafenthiuron 50% WP	362.23 (0.36)	179.70	1043.46	Y=1.88+1.75X	1.75 ± 0.27	0.07
Flonicamid 50% WG	323.42 (0.32)	213.70	734.06	Y=2.35+1.93X	1.93 ± 0.23	0.38
Fipronil 5% SC	312.94 (0.31)	233.74	811.49	Y=2.44+0.94X	0.94 ± 0.24	0.89
Acephate 75% SP	122.90 (0.12)	80.42	177.83	Y=2.43+0.86X	0.86 ± 0.22	0.15
Spinetoram 11.7% SC	441.05 (0.44)	291.27	838.64	Y=2.87+1.04X	1.04 ± 0.23	0.57

Profenofos 50% EC	83.66 (0.08)	51.86	120.53	Y=2.02+0.96X	0.96 ± 0.26	0.63
Imidacloprid 17.8% SL	271.86 (0.27)	173.92	600.61	Y=2.27+1.11X	1.11 ± 0.22	0.40
Lambda-cyhalothrin 5% EC	77.84 (0.07)	43.75	109.14	Y=2.84+0.95X	0.95 ± 0.27	0.50
Azadirachtin 0.03% EC	4642.17 (4.60)	3244.78	6132.75	Y=2.79+1.47X	1.47 ± 0.32	1.13
Imidacloprid 6% + Lambda-cyhalothrin 4% SL	85.81 (0.08)	47.20	132.23	Y=1.96+0.95X	0.95 ± 0.21	0.09
Acephate 50% + Imidacloprid 1.8% SP	73.98 (0.07)	43.22	106.67	Y=2.35+0.99X	0.99 ± 0.20	0.54
Fipronil40%+Imidacloprid 40% WG	208.12 (0.20)	130.58	307.71	Y=2.7+1.11X	1.11 ± 0.26	0.31
Pyriproxyfen 8.0% + Diafenthiuron 30% SE	224.15 (0.22)	131.19	356.96	Y=2.33+1.34X	1.34 ± 0.21	0.21

Table 4: Toxicity response of insecticides against adults of *C. zastrowi sillemi* at 24 hrs after exposure

Insecticides	LC_{50} (ppm or g or ml/L)	Fiducial limit		Regression equation	Slope ± S.E.	χ^2
		Lower	Upper			
Acetamiprid 20% SP	76.90 (0.07)	30.82	126.05	Y=1.61+0.85X	0.85 ± 0.219	0.37
Diafenthiuron 50% WP	252.90 (0.25)	168.57	463.02	Y=2.47+1.03X	1.03 ± 0.223	0.28
Flonicamid 50% WG	227.03 (0.27)	161.37	335.37	Y=2.94+1.25X	1.25 ± 0.244	0.56
Fipronil 5% SC	222.31 (0.22)	124.01	385.70	Y=2.00+0.86X	0.86 ± 0.234	0.22
Acephate 75% SP	70.05 (0.07)	40.10	99.60	Y=2.35+1.27X	1.27 ± 0.249	0.37
Spinetoram 11.7% SC	322.73 (0.32)	205.58	731.42	Y=2.28+0.96X	0.96 ± 0.220	0.34
Profenofos 50% EC	41.94 (0.04)	16.06	66.10	Y=1.55+0.95X	0.95 ± 0.251	0.074
Imidacloprid 17.8% SL	210.50 (0.21)	154.86	312.48	Y=3.01+1.28X	1.28 ± 0.239	0.28
Lambda-cyhalothrin 5% EC	44.04 (0.04)	14.21	74.07	Y=1.45+0.88X	0.88 ± 0.244	0.10
Azadirachtin 0.03% EC	768.41 (0.76)	511.56	992.42	Y=3.03+1.01X	1.01 ± 0.221	0.20
Imidacloprid 6% + Lambda-cyhalothrin 4% SL	54.60 (0.05)	28.09	103.34	Y=1.12+0.64X	0.64 ± 0.213	0.11
Acephate 50% + Imidacloprid 1.8% SP	49.03 (0.04)	7.93	93.97	Y=1.18+0.7X	0.7 ± 0.210	0.21
Fipronil 40% + Imidacloprid 40% WG	110.40 (0.11)	64.82	173.72	Y=1.89+0.99X	099 ± 0.261	0.27
Pyriproxyfen 8.0% + Diafenthiuron 30% SE	196.32 (0.19)	109.41	312.96	Y=2.09+0.91X	0.91 ± 0.218	0.31

Results and Discussion

Ten insecticides and four ready to use combination products were tested for their toxicity to larvae and adults of *C. zastrowi sillemi*. The data on per cent cumulative mortality of larvae (2nd instar) of an *C. zastrowi sillemi* at 24, 48 and 72 hours after exposure to insecticides and adults at 24 hours after exposure are presented hereunder.

Toxicity of insecticides to *C. zastrowi sillemi* grubs

At 24 hours after exposure the lowest toxicity was found in the treatment of azadirachtin 0.03% EC with LC_{50} value of 8142.43 ppm followed by spinetoram 11.7% (1187.98 ppm), flonicamid 50% WG (824.54 ppm), diafenthiuron 50% WP (762.52 ppm), fipronil 5% SC (740.56 ppm) and imidacloprid 17.8% SL (724.50 ppm)

While the highest toxicity was found in treatment with acephate 50% + imidacloprid 1.8% SP with LC_{50} value of 173.90 ppm followed by imidacloprid 6% + lambda-cyhalothrin 4% SL (215.99 ppm), lambda-cyhalothrin 5% EC (220.07 ppm), profenofos 50% EC (241.75 ppm), acephate 75% SP (253.63 ppm) and acetamiprid 20% SP (268.96 ppm) (Table 3). At 48 hours of post treatment lowest toxicity was noticed in the treatment azadirachtin 0.03% EC with LC_{50} value of 6671.59 ppm followed by spinetoram (624.48 ppm), diafenthiuron 50% WP (462.41 ppm), flonicamid 50% WG (445.24 ppm), fipronil 5% SC (412.07 ppm) and imidacloprid 17.8% SL (348.28 ppm). The highest toxicity found in the treatment acephate 50% + imidacloprid 1.8% SP with LC_{50} value of 97.00 ppm fallowed by imidacloprid 6% + lambda-cyhalothrin 4% SL (106.26 ppm), profenofos 50% EC (107.42 ppm), lambda-cyhalothrin 5% EC (119.59 ppm), acephate 75% SP (176.36 ppm) and acetamiprid 20% SP (178.91 ppm), while the lowest toxicity was observed in the treatment of azadirachtin 0.03% EC with LC_{50} value of 6671.59 ppm followed by spinetoram 11.7% SC (724.49 ppm) (Table 4).

At 72 hours of insecticide exposure, lowest toxicity in the treatment of azadirachtin 0.03% EC with LC_{50} value of 4642.17 ppm followed by spinetoram 11.7% SC (441.05 ppm), diafenthiuron 50% WP (362.23 ppm) flonicamid 50% WG (323.42 ppm), fipronil 5% SC (312.94 ppm), imidacloprid 17.8% SL (271.86 ppm). Whereas, the toxicity was more in the treatment acephate 50% + imidacloprid 1.8% SP followed by lambda-cyhalothrin 5% EC, profenofos 50% EC, imidacloprid 6% + lambda-cyhalothrin 4% SL, acephate 75% SP and acetamiprid 20% SP with 73.98, 77.84, 83.66, 85.81, 122.90 and 124.45 ppm, respectively (Table 5).

Irrespective of time of exposure to the insecticides, among the 14 tested insecticides the order of toxicity against grubs of *C. zastrowi sillemi* based on LC_{50} was found highest in the treatment of acephate 50% + imidacloprid 1.8% SP > profenofos > lambda-cyhalothrin 5% EC > imidacloprid 6% + lambda-cyhalothrin 4% SL > acephate 50% + imidacloprid 1.8% SP > acetamiprid 20% SP > acephate 75% SP. Bhojani *et al.* (2018) [4] reported that lambda-cyhalothrin 5% EC and acephate 50% + imidacloprid 1.8% SP were harmful insecticides to the second stage larvae of *C. zastrowi sillemi*. Sabry and Sayed (2011) [9] observed that, lambda-cyhalothrin 5% EC was highly toxic to the second stage larvae of *C. carnea* with LC_{50} value of 0.04 ppm. Shah *et al.* (2012) and Varghese and Beevi (2004) found that profenfos 50% EC was the most toxic to the second instar larvae *C. carnea.*

Toxicity of insecticides to *C. zastrowi sillemi* adults

The data on percent mortality of adults of *C. zastrowi sillemi* recorded at 24 hour after exposure (food contamination technique) revealed that lowest toxicity was recorded in the treatment of azadirachtin 0.03% EC with LC_{50} value of 768.41 ppm followed by spinetoram 11.7% SC (322.73 ppm), diafenthiuron 50% WP (252.90 ppm),

flonicamid 50% WG (227.03 ppm), fipronil 5% SC (222.31 ppm), imidacloprid 17.8% SL (210.50 ppm) while the highest toxicity was recorded in treatment of profenofos 50% EC with LC_{50} value of 41.94 ppm fallowed by lambda-cyhalothrin 5% EC (44.04 ppm), acephate 50% + imidacloprid 1.8% SP (49.03 ppm), imidacloprid 6% + lambda-cyhalothrin 4% SL (54.60 ppm), acephate (70.05 ppm) and acetamiprid 20% SP (76.90 ppm)

The order of toxicity of various insecticides toxicity against the adults of *C. zastrowi sillemi* based on LC_{50} was profenofos 50% EC > lambda-cyhalothrin 5% EC > acephate 50% + imidacloprid 1.8% SP > imidacloprid 6% + lambda-cyhalothrin 4% SL > acephate 75% SP > acetamiprid 20% SP. The present results were in line with the reported that, flonicamid 50% WG was less toxic to adults of *C. carnea*. Sabry and Sayed (2011) [9] reported imidacloprid 70% WG and diafenthiuron 50% WP were found slightly harmful to adults of *C. zastrowi sillemi* when exposed to poisoned food reported that lambda-cyhalothrin was found toxic to adults of *C. johnsoni* and *C. carnea*. Bhojani *et al*. (2018) [4] reported that lambda-cyhalothrin 5% EC and acephate 50%+ imidacloprid 1.8% SP were harmful insecticides to adults of *C. zastrowi sillemi*. Sabry and Sayed (2011) [9] reported that lambda-cyhalothrin 5% EC was highly toxic to the adults of *C. carnea* and they confirmed that adults of *C. carnea* were more susceptible to pesticides than second instar larvae. Nasreen *et al*. (2005) [6] who reported that acetamiprid was highly toxic to adults of *C. zastrowi sillemi*. The higher susceptibility of *C. zastrowi sillemi* grubs and adults to lambda-cyhalothrin could be because of the mode of action of pyrethroids, lambda-cyhalothrin was toxic to both the grubs and adults of *C. zastrowi sillemi* which act functionally on the voltage-sensitive sodium channel. Further, combi-products having lambda-cyhalothrin also found to be toxic because of its higher lipophilicity due to higher Log kow and broad-spectrum activity which make them toxic the natural enemies. Profenofos and acephate were found highly toxic to the grubs and adults. It might be because of lower molecular weight which makes them to penetrate the cuticle and also their long persistency due to oil-based formulation and their ability to inactivate acetylcholinesterase enzyme. Sabry and Sayed (2011) [9]. Acetamiprid found toxic which is having a novel mode of action compared to conventional neurotoxic insecticides. This compound acts on the central and peripheral nervous system of insects, specifically interacts with nicotinic acetylcholine receptors (nAChR), resulting in excitation and paralysis. No previous studies have evaluated the side effects of the insecticide imidacloprid 6% + lambda-cyhalothrin 4% SL on *C. zastrowi sillemi* grubs and adults. The toxicity of combi-product imidacloprid 6% + lambda-cyhalothrin 4% SL on both grubs and adults might be the higher mode of action of lambda-cyhalothrin on insect.

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